

REMARKS

I. Status of the Claims and Formal Matters

Claims 1-95 are pending in the application. Claims 1-3, 10, 12-17 and 19-22 have been amended and claims 8, 11, 36 and 40-95 have been cancelled without prejudice or disclaimer. Accordingly, claims 1-7, 9, 10, 12-35 and 37-39 will be pending in the application upon entry of this paper.

Support for the amendments and new claims is found throughout the application and claims as originally-filed and in the pending claims. In particular, exemplary support for the amendments is as follows. Support for the amendment to step a) of claim 1, which now refers to "low stringency" conditions and binding of the anchor fingers with "low affinity" can be found in original claim 11 and paragraph 73 of U.S. Patent Publication No. 20070178454, which is the official publication of the instant application filed as U.S. Serial No. 10/532,258 on October 2, 2006. Support for the amendment to step c) of claim 1 which now refers to a secondary library "encoding multi-zinc-finger polypeptides having zinc-fingers partially optimized for binding to subsites of the sequence of interest" can be found in paragraph 0138 of U.S. Patent Publication No. 20070178454. Support for the amendment to step d) of claim 1, which now refers to "high-stringency" conditions can be found in original claim 36 and paragraph 73 of U.S. Patent Publication No. 20070178454. Additional amendments to steps b), c), d) and e) of claim 1 have been made to more clearly specify the relationship between the elements of each step and proper antecedent basis can be found within each prior step. Claims 1-3 have also been amended to refer to multi-zinc-finger polypeptides as described in paragraph 59 of U.S. Patent Publication No. 20070178454. Claims 10, 12, 13, 14, 16, 17, and 19 have been amended to more properly refer to "zinc-fingers" rather than zinc fingers. Claims 14, 15, 19 and 20 are amended to refer to synthetic derivatives of Zif268 as described in paragraph 115 of U.S. Patent Publication No. 20070178454. Claims 21 and 22 have been amended to more properly conform to the antecedent basis provided by claim 1.

No new matter has been added by the amendments or new claims.

Amendment and cancellation of the claims herein are not to be construed as acquiescence to any rejections/objections set forth in the pending Office Action and/or any previous Office Actions and were done solely to expedite prosecution of the application. Applicants reserve the right to pursue the claims as originally filed or similar claims in this or one or more subsequent patent applications.

Applicants respectfully request reconsideration and withdrawal of the objections to and the rejections of this application in view of the amendments and remarks herewith, as the application is in condition for allowance.

II. Objections

Claim 8 is objected to under 37 C.F.R. § 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Claim 8 is cancelled by entry of this amendment, thereby obviating the objection.

The oath/declaration is allegedly defective because one of the listed inventors did not provide a signature. The Office's attention is respectfully directed to the petition under 37 C.F.R. § 1.47(a) that was granted on January 30, 2007 regarding the instant application. No additional signature is believed to be required.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing .

III. The Rejections under 35 U.S.C. § 112 are Overcome

Claims 1-39 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants respectfully traverse the rejection.

The Office Action sets forth the allegation that the essential structural cooperative relationship of elements between steps a) and b) are lacking. In particular, the structural cooperative relationship between the element of "isolating pools comprising nucleic acid sequences" of step b) and the various elements of step a) are said to be omitted.

Applicants respectfully invite the Examiner's attention to paragraph 0138 of U.S. Patent Publication No. 20070178454, which states:

The primary selection methods described above will yield a separate "pool" of candidate Zf proteins for each "Zf/subsite" pair. A key aspect of the CSPO strategy is that these "pools" can be recombined to produce a secondary library comprising variants that harbor fingers which have been partially optimized for binding to a desired subsite. For example, such a secondary library can comprise a range of multi-finger proteins composed of random combinations of the pools of fingers selected from the randomized fingers of the primary library.

As used in the context of claim 1, the "pools" are groupings of nucleic acid sequences encoding the multi-zinc-finger polypeptides that have been selected according to the methods of step a). The amendments presented in step b) of claim 1 are intended to clarify further the essential structural cooperative relationship of elements between steps a) and b). Taken together with at least the above-referenced paragraph from the instant specification, Applicants believe that the relationship of elements between steps a) and b) as well as the meaning of the term "pools" would be fully understood by one of skill in the relevant art.

The Office Action sets forth the further allegation that phrases "wherein said polypeptides comprise the first binding complexes" of step b) and "wherein said polypeptides comprise the second binding complexes" of step e) are confusing and unclear. As amended herein, step b) now refers to "multi-zinc-finger polypeptides having one variable finger that formed in the first binding complexes of step a) with the target site constructs" and step e) now refers to "multi-zinc-finger polypeptides that formed in the second binding complexes of step d)" to more clearly specify the elements of the multi-zinc-finger polypeptides referred to at each of step b) and e).

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

IV. The Rejections under 35 U.S.C. § 102 are Overcome

Claims 1-14, 16-19, 21, 22, 27-29, 32, 34, 35 and 37 are rejected under 35 U.S.C. §102(b) as being anticipated by Isalan, *et al.* (Nat Biotechnol. 2001 Jul;19(7):656-60; "Isalan"). Applicants respectfully disagree and traverse the rejection.

To form the basis of a proper rejection under 35 U.S.C. § 102(b), a cited reference must disclose each and every element of the rejected claim. *See Lewmar Marine Inc. v. Barient Inc.*, 3 U.S.P.Q.2d 1766 (Fed. Cir. 1987) and Manual of Patent Examining Procedure (MPEP) § 2131. Applicants respectfully submit that Isalan fails to disclose one or more elements of the invention, as claimed herein, and therefore, should be removed from consideration under 35 U.S.C. § 102(b).

As amended herein, claim 1 is now recites a first low-stringency selection step as recited in step a) and a second high-stringency selection step as recited in step d). Isalan does not teach or suggest a two part method having a first low-stringency selection step followed by a second high-stringency selection step. In addition, as discussed at length below, Isalan employs 1.5 variable fingers in its methods while the claimed methods employ only a single variable finger. Isalan fails to teach each and every element of the claimed invention and, therefore, does not anticipate the presently claimed invention.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 102(b).

V. The Rejections under 35 U.S.C. § 103 are Overcome

Claims 1-14, 16-19, 21-25, 27-29, 32, 34, 35, and 37 are rejected under 35 U.S.C. §103(a) as being unpatentable over Isalan, *et al.* (Nat Biotechnol. 2001 Jul;19(7):656-60; "Isalan") in view of Isalan, *et al.* (Biochemistry. 1998 Sep 1;37(35):12026-33; "Isalan II"). Applicants respectfully disagree and traverse the rejection.

In order to make out a *prima facie* showing of obviousness, the Office Action must provide some motivation to combine the references, the combination of references must teach or suggest each and every element of the claimed invention, and there must be some reasonable expectation of success in making and using the invention as claimed. (MPEP §2141). In the present case, the references cited in the Office Action fail to teach or suggest all of the claim limitations; fail to provide the requisite motivation to combine; and fail to provide a reasonable expectation of success.

Claim 1 has been amended to recite “incubating ... under low-stringency conditions” in step a) and “incubating ... under high-stringency conditions” in step d); and to recite that the multi-zinc-finger polypeptides have “one variable finger.” Thus, the invention as currently claimed includes a first round of low-stringency selection using maximally randomized libraries in which a single variable finger has been randomized followed by a second round of high-stringency selection.

As detailed below, none of the cited references, alone or in any combination, teaches or suggests that two rounds of selection should be carried out; more specifically, none teaches that a first round of low-stringency selection should be followed by a second round of high-stringency selection; furthermore, none teaches that the low stringency selection should employ randomized libraries in which a single variable finger has been randomized. Thus, none of the references, alone or in any combination, supports the obviousness rejection of the claims.

Isalan

In contrast to Applicants' invention as presently claimed, Isalan teaches a selection method that involves a single high stringency screening step. Specifically, Isalan teaches that the high-stringency selection of the two libraries is carried out in parallel against DNA sequences in which either the first or the last 5 bp of the 9 bp Zif268 target site are replaced with the corresponding bases from a target site of interest. After phage display selection, pools of binding fingers from the two prefabricated libraries are recombined to produce a partially selected library of three finger proteins, which are selected against the full 9 bp sequence of interest. In sum,

Isalan merely describes a single high stringency selection. Isalan neither teaches nor suggests using a two step process of low-stringency selection followed by a high-stringency selection, as Applicants presently claim.

Additionally, methods of the invention being claimed advantageously employ just one variable (i.e., randomized) zinc-finger. By contrast, Isalan describes randomizing 1.5 fingers out of the three Zf protein Zif268 in each library, at residues in the recognition helices of spanning 1.5 fingers (see, e.g., the section entitled "The 'bipartite' library strategy." pages 656-657 and Figure 2). In this regard, the Isalan selection method suffers from limited combinatorial diversity in its two libraries.

Because the Isalan libraries each encompasses more than one variable finger, the number of amino acid residue positions that must be randomized is greater than six residues (the number of DNA-binding positions in a single finger). Specifically, the libraries described by Isalan require randomization of 8 or 10 total residue positions.

The selection techniques used by Isalan to engineer zinc-fingers require passage of the library DNA through bacteria (e.g. phage display, bacterial cell-based selections). Thus, the transformation efficiency of bacteria places a limit on the size and the representation of a library made in this manner. Because the highest available transformation efficiency of bacteria is limited to $\sim 10^9$ transformants per aliquot of DNA per transformation event, the representation of a single library that can be created according to the Isalan method is also limited to $\sim 10^9$ candidates, at best.

Therefore, the transformation efficiency of bacteria sets a limit on the number of positions that can be varied in Zf proteins and on the representation of a library attempting to contain all possible randomized combinations of Zf proteins. Even using the most efficient techniques for generating combinatorial libraries (where each of the potential 20 amino acids is encoded by a single codon), one can only create fully saturated, randomized libraries in bacteria where at most six amino acid positions are varied (six variable positions: $20^6 = 6.4 \times 10^7$ vs. seven variable positions: $20^7 = \sim 1.3 \times 10^9$).

Thus, the libraries described by Isalan possess a severe limitation in the number of amino acids that can be used at each randomized position (see, e.g., Figure 2b of

Isalan). Because of this limitation, the initial libraries of Isalan exclude many potential zinc-finger variants, including those that would optimally bind a target site of interest.. Accordingly, such optimal fingers may not even be present in the initial libraries, and would not be identified by practicing the methods of Isalan.

In sum, Isalan fails to describe the invention as presently claimed because Isalan fails to teach a selection method that involves a two-step screening process, fails to describe low and high stringency screening, and fails to teach methods that employ one variable (*i.e.*, randomized) zinc-finger.

Isalan II

The Office seeks to remedy the deficiencies of Isalan by citing Isalan II, but this reliance on Isalan II is unavailing because Isalan II fails to teach or suggest a two-step screening process that involves carrying out a low-stringency selection prior to a high-stringency selection. The combination of Isalan II with Isalan does not direct one of skill in the art to employ a first round of low-stringency selection using a single variable finger followed by a second round of high-stringency selection.

Additionally, Isalan II teaches the randomization of amino acid positions in adjacent zinc fingers that specify overlapping DNA subsites (Abstract). Thus, randomized amino acid positions spanning two zinc fingers are screened under high-stringency selection. In contrast, Applicants' methods involves low-stringency selection of libraries containing randomized amino acid residues in a single variant zinc finger. In sum, Isalan II does not make up for the deficiencies in Isalan.

Both Isalan and Isalan II direct one of skill in the art to use a single high-stringency selection method. This bias for using only high-stringency selection taught by Isalan and Isalan II teaches away from the claimed methods. Based on this teaching away, what motivation would one of ordinary skill in the art have to use a first round of low-stringency sections? None whatsoever.

Applicants note that M.P.E.P. 2144.05 states that "[a] prima facie case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention." *In re Geisler*, 116 F.3d 1465, 1471 (Fed. Cir.

1997). Applicants submit that in view of this clear teaching away, one of ordinary skill in the art would not be motivated to combine Isalan and Isalan II.

However, ***assuming for the sake of argument*** there were some motivation to combine Isalan and Isalan II, the combination would not provide each and every element of the claims presented herein because of the deficient teachings of the references as detailed above. Furthermore, again, ***assuming for the sake of argument*** that there were some motivation to combine the references and that the combination provided each and every element of the claims, the clear teaching away from the claimed invention taught in the cited references would not provide a reasonable expectation of success to one of ordinary skill in the art.

Accordingly, Applicants respectfully submit that the Office Action has failed to make out a *prima facie* showing of obviousness and request reconsideration and withdrawal of the rejection of claims 1-14, 16-19, 21-25, 27-29, 32, 34, 35, and 37 under 35 U.S.C. §103(a) over Isalan and Isalan II.

Claims 1-14, 16-19, 21-29, 32-35 and 37 are rejected under 35 U.S.C. §103(a) as being unpatentable over Isalan, *et al.* (Nat Biotechnol. 2001 Jul;19(7):656-60; "Isalan") in view of Isalan, *et al.* (Biochemistry. 1998 Sep 1;37(35):12026-33; "Isalan II"), and further in view of Choo (WO 2000/27878). Applicants respectfully disagree and traverse the rejection.

The claims as currently amended recite "incubating ... under low-stringency conditions" in step a) and "incubating ... under high-stringency conditions" in step d). As indicated in the foregoing remarks, Isalan and Isalan II, alone or in combination, do not teach or suggest a first round of low-stringency selection using maximally randomized Zf libraries followed by a second round of high-stringency selection.

Isalan and Isalan II were discussed above and Applicants reiterate that discussion here.

Choo

To remedy the deficiencies of Isalan and Isalan II, the Office Action cites Choo (WO 2000/27878). Applicants submit that Choo fails to remedy these deficiencies.

In contrast to Applicants' claimed methods, Choo describes a method for producing a zinc finger nucleic acid binding protein by selecting variants which bind to a target nucleic acid sequence by polysome display (Table 1). Choo does not teach Applicants' claimed two-step process, which involves high stringency and low stringency selections (page 19, lines 18-27). Rather, the *in vitro* methods taught by Choo are specific to polysome display. Choo does not teach adjusting the stringency of selection in polysome display methods by varying salt concentration (e.g., high salt conditions) or temperature (e.g., between 37°C and 47°C), as Applicants do. Therefore, Choo does not teach a low-stringency selection prior to a high-stringency selection according to the *in vitro* selection methods being claimed, and does not make up for the deficiencies of Isalan and Isalan II. Moreover, adding the teachings of Choo to the combination of Isalan and Isalan II still would not provide each and every element of the claims presented herein.

As noted above, the bias for using only high-stringency selection taught by Isalan and Isalan II clearly teaches away from the claimed methods. Applicants note that M.P.E.P. 2144.05 states that "[a] prima facie case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention." *In re Geisler*, 116 F.3d 1465, 1471 (Fed. Cir. 1997). Applicants submit that in view of this clear teaching away, one of ordinary skill in the art would not be motivated to combine Isalan and Isalan II and would not have a reasonable expectation of success. Addition of a third reference (Choo) does not rehabilitate the failure of the primary and secondary references to establish a *prima facie* case of obviousness.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-14, 16-19, 21-29, 32-35 and 37 under 35 U.S.C. §103(a) over Isalan and Isalan II further in view of Choo.

Claims 1-14, 16-19, and 21-39 are further rejected under 35 U.S.C. 103(a) as being unpatentable over Isalan, Isalan II, and Choo in further view of Joung, *et al.* (Proc Natl Acad Sci U S A. 2000 Jun 20;97(13):7382-7; "Joung"). As an initial matter, Applicants have cancelled claim 36, thereby rendering the rejection moot as to claim 36. With regard to the remaining claims, Applicants respectfully disagree and traverse the rejection.

The claims as currently amended recite "incubating ... under low-stringency conditions" in step a) and "incubating ... under high-stringency conditions" in step d). As indicated in the foregoing remarks, Isalan, Isalan II, and Choo, alone or in any combination, do not teach or suggest a first round of low-stringency selection using maximally randomized Zf libraries prior to a second round of high-stringency selection.

Isalan, Isalan II and Choo were discussed above and Applicants reiterate that discussion here.

Joung

Joung discloses a bacterial "two hybrid" system that readily allows selection from libraries larger than 10^8 in size (Abstract). Joung describes the use of their "two hybrid" system to select zinc finger variants (from a large randomized library) that bind tightly and specifically to desired DNA target sites. Nevertheless, the selections for zinc finger variants taught by Joung all involve only a single round of selection employing one level of stringency. For example, practicing the method of Joung would exclude steps c) – e), as recited in Claim 1, from which claims 2-14, 16-19, and 21-39 depend. In fact, Joung states: "...[our method] permits functional fingers to be isolated in a single selection step instead of using multiple rounds of enrichment" (at page 7387, second full paragraph, emphasis added) and "... our selection system permits the single-step isolation of candidates in an *in vivo* context ..." (at page 7387, last paragraph before the acknowledgements; emphasis added).

Clearly, Joung fails to describe Applicants' claimed selection methods. Adding the teachings of Joung to the other references cited in rejection still would not provide each and every element of the claims presented herein. Moreover, Joung teaches

away from a method as Applicants currently claim involving two selection steps with different levels of stringency because Joung teaches that success can be attained in a single round of selection. Based on the Joung teachings, what motivation would one of ordinary skill in the art have to go to a two selection step system? None whatsoever.

As noted above, the bias for using only high-stringency selection taught by Isalan and Isalan II clearly teaches away from the claimed methods. Joung provides a second teaching away inasmuch as Joung teaches that success can be attained in a single round of selection whereas Applicants' claims provide for two selection steps with different levels of stringency. Applicants again note that M.P.E.P. 2144.05 states that "[a] *prima facie* case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention." *In re Geisler*, 116 F.3d 1465, 1471 (Fed. Cir. 1997).

Just as addition of a third reference (Choo) did not rehabilitate the failure of the primary and secondary references to establish a *prima facie* case of obviousness, the addition of a fourth reference (Joung) does not remedy the situation. Moreover, Joung only exacerbates the failure with an additional clear teaching away. With the teachings away of Isalan, Isalan II and Joung, one of ordinary skill in the art most certainly would not be motivated to combine the references (such a combination would not teach all the elements of the claims) and would not have a reasonable expectation of success.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-14, 16-19, and 21-39 under 35 U.S.C. §103(a) over Isalan, Isalan II and Choo further in view of Joung.

Claims 1-39 are further rejected under 35 U.S.C. 103(a) as being unpatentable over Isalan, Isalan II, Choo, and Joung in further view of Chandrasegaran, *et al.* (US 6,265,196; "Chandrasegaran") As an initial matter, Applicants have cancelled claim 36, thereby rendering the rejection moot as to claim 36. With regard to the remaining claims, Applicants respectfully disagree and traverse the rejection.

The claims as currently amended recite "incubating ... under low-stringency conditions" in step a) and "incubating ... under high-stringency conditions" in step d). As

indicated in the foregoing remarks, Isalan, Isalan II, Choo and Joung, alone or in any combination do not teach or suggest a first round of low-stringency selection using maximally randomized Zf libraries prior to a second round of high-stringency selection.

Isalan, Isalan II, Choo and Joung were discussed above and Applicants reiterate that discussion here.

Chandrasegaran

Chandrasegaran teaches methods for enzymatically inactivating a target DNA, detecting conformational change in a nucleic acid, and detecting the presence of a target DNA molecule (Abstract). Regarding the specific zinc finger motifs recited in claims 15 and 20, Chandrasegaran teaches that these particular sequences are present in zinc finger proteins, which bind DNA (column 2, lines 39-49), but does not describe selecting zinc fingers using a method involving a low-stringency selection followed by a high-stringency selection. Therefore, Chandrasegaran does not remedy the deficiencies of Isalan, Isalan II, Choo, and Joung.

As noted above, the bias for using only high-stringency selection taught by Isalan and Isalan II clearly teaches away from the claimed methods. Joung provides a second teaching away inasmuch as Joung teaches that success can be attained in a single round of selection whereas Applicants' claims provide for two selection steps with different levels of stringency. Applicants once again note that M.P.E.P. 2144.05 states that "[a] *prima facie* case of obviousness may also be rebutted by showing that the art, in any material respect, teaches away from the claimed invention." *In re Geisler*, 116 F.3d 1465, 1471 (Fed. Cir. 1997).

Just as addition of a third reference (Choo) and a fourth reference (Joung) did not rehabilitate the failure of the primary and secondary references to establish a *prima facie* case of obviousness, the addition of a fifth reference (Chandrasegaran) does not remedy the situation. Moreover, with the teachings away of Isalan, Isalan II and Joung, one of ordinary skill in the art most certainly would not be motivated to combine the references (such a combination would not teach all the elements of the claims) and would not have a reasonable expectation of success.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of Claims 1-39 under 35 U.S.C. §103(a) over Isalan, Isalan II, Choo and Joung further in view of Chandrasegaran.

On a final note, Applicants submit that to properly determine a *prima facie* case of obviousness, the Examiner “must step backward in time and into the shoes worn by the hypothetical ‘person of ordinary skill in the art’ when the invention was unknown and just before it was made.” M.P.E.P § 2142. This is important as “impermissible hindsight must be avoided and the legal conclusion must be gleaned from the prior art.” *Id.*

Applicants submit that the foregoing obviousness rejections are nothing more than a hindsight reconstruction of the claimed invention ***based on Applicants’ own teachings***. Although an Examiner often uses hindsight in formulating an obviousness rejection, such use of hindsight is permitted as long as the legal conclusion is ***gleaned from the prior art and not from Applicants’ teachings***. The rejections are nothing more than the picking and choosing of claim elements from multiple references using Applicants’ teachings as a blueprint.

As noted above, there is no motivation in the cited art to combine the references and, even if combined, the references in combination do not disclose all the elements of the claims. Moreover, in view of the clear teachings away from the claimed invention in the two primary references (Isalan and Isalan II) and in one of the secondary references (Joung), one of ordinary skill in the art would not only have no motivation to combine the references but also would lack any reasonable expectation of success based on those teachings.

VI. The Rejection based on Obviousness-Type Double-Patenting is Overcome

Claim 1 is provisionally rejected on the ground of non-statutory obviousness-type double-patenting as being unpatentable over claims 51 and 52 of co-pending Application No. 10/532,031. It is respectfully submitted that co-pending Application No. 10/532,031 was abandoned on July 21, 2009 for failure to respond to an outstanding

Office Action (*i.e.*, Restriction Requirement). Reconsideration and withdrawal of the non-statutory obviousness-type double-patenting rejections are respectfully requested.

CONCLUSION

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of all objections and rejections and allowance of the application with all pending claims.

Applicants gratefully acknowledge the Examiner's willingness to participate in an interview, as set forth on page 2 of the Office Action. Accordingly, if the Examiner is not inclined to allow the application upon consideration of this paper, Applicants respectfully invite the Examiner to contact the undersigned so that a telephonic interview with Examiner can be scheduled prior to issuance of the next Office Action.

Dated: October 1, 2009

Respectfully submitted,

Electronic signature: /Melissa Hunter-Ensor/
Melissa Hunter-Ensor, Esq.

Registration No.: 55,289
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5509
Attorneys/Agents For Applicants